Pro-Inflammatory Levels of Interleukin-1α-Like Bioactivity Are Present in the Majority of Open Comedones in Acne Vulgaris

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The factors that initiate the inflammatory response in acne are not known. The presence of pro-inflammatory cytokines in acne comedones was therefore investigated.

One hundred eight open comedones were collected from 18 untreated acne patients (10 male, 8 female). Each comedone was homogenized and centrifuged, and the supernatant was analyzed for bioactive and immunochemically detectable IL-1α, IL-1β, and TNFα. Viable counts of propionibacteria, staphylococci, and *Malassezia* spp. were determined in the comedone pellet.

Bioactive IL-1α-like material was demonstrated in 76% of open comedones (range of 23–4765 pg IL-1α-like bioactivity/mg of comedone material). In 58% of comedones, levels exceeded 100 pg/mg. There was no correlation between IL-1α-like bioactivity and IL-1α determined immunochemically.

Bioactive IL-1β was not detected in any comedones. Twenty-four percent contained low levels of immunochemical IL-1β (range 12–103 pg IL-1β/mg comedone material). Bioactive TNFα was detected in three comedones with a further five comedones containing immunochemical TNFα (range of 61–820 pg TNFα/mg comedone material).

The majority of open comedones (97%) contained microorganisms. There was, however, no significant correlation (Spearman's rank) between levels of any cytokine, in particular IL-1α-like bioactivity, and numbers of microorganisms.

Thus, bioactive IL-1α-like material in the majority of open comedones may be concerned in the initiation of inflammation in acne following spongiosis or rupture of the pilosebaceous follicle wall. *J Invest Dermatol 98:895–901, 1992*

The pathogenesis of acne vulgaris is still not fully understood despite decades of research. The two major clinical lesions are the non-inflamed comedones and the clinically more significant inflamed lesions. The interrelationship between these two types of lesion is uncertain but the available evidence favors the concept that inflamed lesions arise from non-inflamed comedones [1], which are the clinical manifestations of ductal hypercornification.

Several factors have been suggested as mediators of inflammation. Microorganisms (especially *Propionibacterium acnes*) and their products [2,3] have been strongly implicated because treatments that reduce their numbers are usually therapeutically successful [4–6].

There has been controversy concerning the initial cellular infiltrate in the developing inflamed lesion. Lynch [7] and Vasarinsh [8] favored the lymphocyte, whereas Kligman [1] stated that the neutrophil was the initial cell to appear. Kligman's observations have profoundly influenced research in the acne field, which has attempted to take account of the neutrophil in the initial development of the inflammatory response [3,9–12]. More recent evidence supports the earlier contention that the initial infiltrate is lymphocytic. In the only reported study of "timed" lesions, undergoing inflammatory changes, Norris and Cunliffe [13] showed that the initial infiltrate in 100% of developing inflammatory lesions was mononuclear (predominantly CD4+ve T lymphocytes). Thus, inflammatory acne, in common with other dermatoses such as psoriasis [14] and contact dermatitis [15], involves non-random helper T-lymphocyte infiltration.

Current researches into the mechanisms of cutaneous inflammation have indicated important unifying concepts [16]. It has been proposed that T lymphocytes will migrate, independently of antigen, into the dermis following the release of pro-inflammatory cytokines by keratinocytes in response to environmental stimuli [15]. The initiation of antigen-independent cutaneous inflammation may facilitate or promote an amplification phase via antigen-dependent T-lymphocyte responses [15]. To date, no studies have reported upon pro-inflammatory cytokine expression in acne vulgaris. Because both T-lymphocyte infiltration and keratinocyte dysfunction have been implicated in the pathogenesis of early inflamed lesions of acne vulgaris, a potential framework exists whereby cytokines might regulate the inflammatory response. The

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Abbreviations:
CD: cluster of differentiation
cfu: colony-forming unit
CV: coefficient of variation
DMEM: Dulbecco's modified Eagle's medium
ELISA: enzyme-linked immunoadsorbent assay
FCS: fetal calf serum
IFN: interferon
IL: interleukin
IU: international unit
MTT: 3-(4,5-dimethyl thiazol-2-yl)-2,5 diphenyl tetrazolium bromide
pI: isoelectric point
RPMI: Rosslyn Park Memorial Institute
TNF: tumor necrosis factor

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aims of this study were to determine the levels of bioactive pro-inflammatory cytokines in acne comedones and to determine whether there was any correlation between the cytokine content of individual lesions and their microflora.

MATERIALS AND METHODS

Reagents RPMI-1640, Dulbecco's modified Eagle's medium (DMEM), Hepes, fetal calf serum, 2-mercaptoethanol (50 mM), and $NaHCO_3$ (7.5%) were obtained from Gibco. Benzyl penicillin sodium B.P. was obtained from Glaxo and streptomycin sulphate was from Evans. MTT [3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl tetrazolium bromide] was from Sigma. L929 cells were obtained from the European collection of animal cell cultures (Porton Down, U.K.). Brain Heart Infusion agar was supplied by Difco. All other components for microbial culture media were from Oxoid Ltd. L-glutamine was obtained from Flow Labs. All tissue culture plastics were cell culture grade from Falcon. All other reagents were supplied by British Drug Houses.

Cytokines and Antibodies Human recombinant IL-1α, IL-1β, IL-3, IL-4, IL-6, TNFα, and TNFβ were obtained from British Biotechnology. Human recombinant IL-2 and IFNγ were from Boehringer. Rabbit polyclonal neutralizing antibodies specific for IL-1α, IL-1β, and TNFα were obtained from British Biotechnology.

Patients Open comedones were donated by 18 acne patients, 10 males (age, 13 to 26 years) and eight females (age, 15 to 26 years). The acne severity ranged from mild to severe. No patients had received any therapy or any natural UV irradiation for 6 weeks. Three males and three females were sampled from the face with the remainder being sampled from the back.

Recovery and Extraction of Comedones Six open comedones were collected aseptically from each patient using a comedone extractor (Thackray) after swabbing the skin surface with isopropanol. No sample was obviously contaminated with blood. The wet weight of each comedone was determined prior to gently homogenizing for 1 min in a micro-tissue homogeniser (Thackray) in 250 μl of DMEM supplemented with 2 mM L-glutamine, 0.375% (w/v) $NaHCO_3$, 20 mM Hepes, and 10% (v/v) FCS (base medium). The homogenate was centrifuged (10,000 $\times g$; microfuge; MSE Microcentaur) for 10 min. The supernatant (approximately 230 μl) was removed and the pellet resuspended in 200 μl of wash fluid (0.075 M sodium phosphate buffer containing 0.1% [v/v] Triton-X100; pH 7.9) for microbiologic determinations.

One hundred microliters of the supernatant was diluted 1:5 with base medium containing penicillin (100,000 U/l) and streptomycin (100 mg/l). One aliquot (420 μl) was immediately utilized for the determination of bioactive TNFα. The remaining 80 μl was stored at $-20°C$ for the determination of immunochemical TNFα. One hundred thirty microliters of the supernatant was diluted 1:10 with base medium plus antibiotics, aliquotted, and stored at $-20°C$ for the determination of bioactive and immunochemical IL-1α and β.

IL-1 Bioassay This was adapted from the method of Falk et al [17]. The assay measured the response of murine (C3H Hej) thymocytes stimulated by IL-1 in the presence of saturating levels of IL-2. Thymocytes were obtained from freshly sacrificed 4- to 8-week-old mice using standard methodology [18]. A single-cell suspension of thymocytes was adjusted to 4×10^7 ml in RPMI-1640 supplemented with 100,000 U penicillin/l; 100 mg streptomycin/l; 5% (v/v) FCS (Myoclone plus); 2.5×10^{-5} M 2-mercaptoethanol; 2 mM L-glutamine; 20 mM Hepes; and 15 IU human recombinant IL-2/ml (IL-1 culture medium, IL-1 CM). Fifty-microliter aliquots of the thymocytes were dispensed into wells of flat-bottomed 96-well microplates.

Comedone supernatants previously diluted 1:10 (see above) were divided into 4×200 μl aliquots; received 25 μl of anti-IL-1α (100 μg/ml) plus 25 μl of IL-1 CM, received 25 μl of anti-IL-1β (100 μg/ml) plus 25 μl of IL-1 CM, received 25 μl anti-IL-1α plus 25 μl of anti-IL-1β, and received 50 μl of IL-1 CM. Each sample was incubated at 37°C for 1 h, doubly diluted eight times in IL-1 CM, and 50 μl of each dilution were added to duplicate wells together with the thymocytes.

Microplates were incubated at 37°C for 72 h in a humidified atmosphere of 5% (v/v) CO_2 in air. Each well was then pulsed with 10 μl of MTT (5 mg/ml in PBS; pH 7.5) and incubated for a further 4 h. One hundred microliters of SDS (100 g/l in 0.01 N HCl) was then added to each well, plates incubated overnight at 37°C in a moist box, mixed, and the optical density of each well at 610 nm determined using an ELISA plate reader.

All assays were standardized against both the reference IL-1α and IL-1β human recombinant international standards [National Institute of Biological Standards and Controls (NIBSC), South Mimms, U.K.]. Standard IL-1 concentration response curves were obtained using a range of concentrations from 0.75–200 ng/l in triplicate. One ng of each standard was approximately equivalent to 100 units of IL-1 bioactivity. The dilution of each test sample and standard giving 50% of the maximum response was determined by Probit analysis. The levels of IL-1α and IL-1β–like activity in the samples were then calculated as ng/l by comparison with the relevant standard.

Human recombinant IL-2, IL-3, IL-4, IL-6, TNFα, TNFβ, and IFNγ did not demonstrate any activity in the assay at concentrations from 0.15 to 5 μg/l. No synergistic or antagonistic effects of these cytokines were observed when tested at 1 μg/l with a range of human recombinant IL-1β concentrations from 0.01 to 2 μg/l. The bioassay detected 1.5 ng IL-1α and β/l. Allowing for the dilution of the comedone supernatant the sensitivity was approximately 10 pg/comedone. The inter-assay variability (CV) determined with 100 ng/l human recombinant IL-1β/l was 26%.

TNF Bioassay A conventional L929 cytotoxicity assay was used. L929 cells in late logarithmic growth were adjusted to 2.5×10^5/ml in DMEM supplemented with 2 mM-L-glutamine, 0.375% (w/v) $NaHCO_3$, 10% (v/v) FCS, 100,000 U penicillin/l, and 100 mg streptomycin/l (TNF CM). Fifty-microliter aliquots were dispensed into wells of flat-bottomed 96-well plates and allowed to adhere at 37°C in a humidified atmosphere of 5% (v/v) CO_2 in air for 3 h.

Comedone supernatants, previously diluted 1:5 were divided into 2 aliquots. One aliquot (210 μl) received 10 μl of anti-TNFα (100 ng/ml) and the second (210 μl) received 10 μl of TNF CM. The samples were incubated at 37°C for 1 h, doubly diluted in TNF CM eight times, and 50 μl of each dilution were added to duplicate wells containing L929 cells. Plates were incubated at 37°C in a humidified atmosphere of 5% (v/v) CO_2 in air. After 72 h, the viability of the cells was determined by MTT conversion as described above for the IL-1 bioassay. All assays were standardized using the interim human recombinant TNFα reference standard (NIBSC). Standard TNFα response curves were obtained using a range of concentrations from 3.5–400 ng/l. The dilution of each sample and standard giving 50% cytotoxicity was determined by Probit analysis. The TNFα levels in the samples were calculated in ng/l by comparison with the standard. One nanogram of TNFα was approximately equal to 40 units of TNFα bioactivity.

Human recombinant IL-1α, IL-1β, IL-2, IL-3, and IFNγ did not demonstrate any activity in this assay at concentrations from 0.15 to 5 μg/l. Human recombinant IL-4 and IL-6 demonstrated significant cytotoxicity at 1.25 and 5 μg/l, respectively ($p < 0.05$; 95% confidence limits). TNFβ demonstrated greater cytotoxicity than TNFα at equivalent concentrations. No synergistic or antagonistic effects of IL-1α, IL-1β, IL-2, IL-3, IL-4, IL-6, or IFNγ were observed when tested at 1 μg/l with a range of 0.03–4 μg TNFα/l.

The assay would detect 15 ng human recombinant TNFα/l provided that L929 cells of less than 15 passages were utilized. Allowing for the dilution of the comedone supernatant, the lower level of sensitivity was approximately 50 pg TNFα/comedone. The inter-

assay variability (CV) estimated with 250 ng/l human recombinant TNFα was 14.9%.

Cytokine Immunochemical Assays Immunochemical IL-1α and TNFα were determined using Quantikine ELISA kits (British Biotechnology). IL-1β was determined using Cistron enzyme-linked immunoadsorbent assay (ELISA) kits supplied by T-Cell Sciences. All assays were carried out according to the manufacturers' instructions. These ELISA were not validated "in house." The sensitivity of the IL-1α kits varied from 17 to 31 ng/l (170-310 pg IL-1α/comedone). For the IL-1β ELISA the sensitivity was 4 ng/l (20 pg IL-1β/comedone). TNFα kits were able to detect 15 ng/l (90 pg TNFα/comedone).

Enumeration of Microorganisms Bacteria and yeasts were determined in the pellet from the centrifugation of comedonal homogenates after resuspension in wash fluid. Viable counts [expressed as colony-forming units (cfu)] were obtained by plating out serial tenfold dilutions of the wash fluid onto a) Brain Heart Infusion agar, incubated anaerobically at 37°C for 7 d, for the isolation of propionibacteria; b) heated blood agar, incubated aerobically at 37°C for 2 d, for the isolation of staphylococci, and c) milk agar [19], at 34°C for 14 d in a moist chamber for isolation of *Malassezia (Pityrosporum)* spp. The lower limit of detection was 4 cfu-comedone. Recovery of a "color-tagged" staphylococcus from freshly isolated comedones homogenized in wash fluid after prior homogenization in base medium demonstrated no adverse effects of the procedure. Wash fluid was routinely utilized for total microscopic microbial counts to ensure the validity of viable counts. Propionibacteria were speciated according to the method of Marples and McGinley [20]. Isolates of staphylococci were identified using API Staph strips.

Statistical Analyses These were carried out according to the recommendations of Sokal and Rohlf [21]. Spearman rank correlation analyses were carried out using Statsoft (Microsoft Corp).

RESULTS

IL-1 Content of Comedones Preliminary studies were carried out to determine whether comedone material interfered with the IL-1 bioassay and the stability of IL-1 in comedone material upon storage. Pooled comedones were "spiked" with IL-1 culture medium (CM) alone, IL-1 CM containing 1 μg, or 0.5 μg IL-1β/l. Bioactive IL-1 was then determined at time zero, after 7 d, and after 14 d of storage at −20°C. At time zero, 0, 1.15 μg IL-1β/l, and 0.59 μg IL-1β/l were recovered. At 14 d, 128% and 70% of the "spiked" levels were estimated. Thus, the IL-1 bioassay was not adversely affected by the comedone material, the added IL-1β was not inhibited and was acceptably stable upon storage for up to 2 weeks at −20°C.

One hundred six comedones were assayed for IL-1 bioactivity. In order to determine the species of IL-1 present, each comedone supernatant was assayed after neutralization with anti-IL-1α and anti-IL-1β. In addition, supernatants assayed without neutralization and after neutralization with both anti-IL-1α and anti-IL-1β served as controls for assay specificity. A typical result for one individual comedone is presented in Fig 1. When IL-1 bioactivity was detected it was always totally neutralized by anti-IL-1α but not by anti-IL-1β alone. The IL-1 bioactivity in comedones is henceforth referred to as IL-1α-like. This activity was demonstrated in 76% of the comedones tested with levels ranging from 23 to 4765 pg/mg of comedone material (14-2725 pg IL-1α-like bioactivity/comedone). Fifty-eight percent of comedones contained over 100 pg IL-1α-like bioactivity/mg comedone material. The results obtained for each individual are shown in Fig 2. There was no evidence that the site (back or face) or sex was preferentially associated with the presence of IL-1α-like bioactivity. A total of 108 comedones were assayed for IL-1α and IL-1β by ELISA. Only 11 had immunochemically detectable IL-1α in the range 119 to 427 pg/mg comedone material (170-427 pg/comedone). Twenty-six comedones contained IL-1β in the range 12-103 pg/mg comedone material (17-64 pg/comedone). There was no correlation between the estimated levels of IL-1α-like bioactivity and immunochemically detectable IL-1α (Fig 3). It was not possible to correlate bioactive and immunochemical IL-1β, because no bioactive IL-1β-like activity was demonstrated in any of the comedones tested.

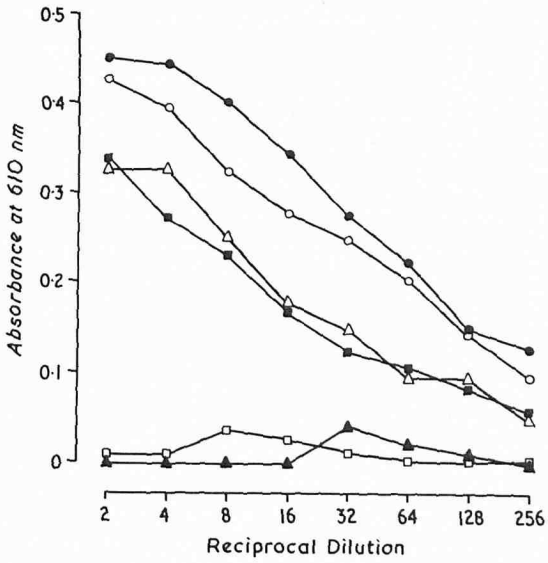

Figure 1. Dilution-related IL-1 activity induced by a comedone extract (△), after neutralization with anti-IL-1α (□), after neutralization with anti-IL-1β (■), and after neutralization with both anti-IL-1α and β (▲). *Closed circles* and *open circles* represent dilutions of 400 ng human recombinant IL-1α and IL-1β international standards/l are recognized by ● and ○, respectively. *Points* show the means of triplicate (standards) or duplicate (comedone extracts) determinations in the C3H thymocyte bioassay for IL-1 in the presence of saturating IL-2.

TNFα Content of Comedones Pooled comedones were "spiked" with human recombinant TNFα to a concentration of 20, 10, 2, and 0 μg/l of comedone material. These were bioassayed at time zero, 7 d, and 14 d after storage at −20°C. At time zero, 22, 11.4, 2.39, and 0 μg TNFα bioactivity/l were recovered. After 1 week at −20°C, approximately 70% activity remained in the "spiked" material. This bioactivity had declined to approximately 15% by 14 d. Thus, although comedone material did not adversely affect the determination of bioactive TNFα. TNFα was unstable upon storage at −20°C in comedone material. (Stability was maintained in culture medium.) Hence, all TNFα bioassays were carried out directly on fresh comedone supernatants within 2 h of collection.

A total of 108 comedones were assayed for bioactive TNFα. All bioassays were carried out with and without prior neutralization with anti-TNFα antibodies to confirm specificity. Bioactive TNFα was detected in only three comedones. The levels were 61, 283, and 820 pg TNFα/mg comedone material (37, 28, and 82 pg/comedone). All three of these comedones were isolated from the back of one female patient. TNFα was also measured by ELISA and five comedones contained detectable, immunochemical TNFα. The levels ranged from 156 to 390 pg TNFα/mg comedone material (91-294 pg/comedone). These five comedones were additional to the three with bioactive TNFα, in which the estimated TNFα levels/comedone were below the sensitivity of the ELISA.

Microbial Content of Comedones The data for the distribution of microbial densities in comedones is summarized in Fig 4. Eighty-three percent of the comedones tested contained propionibacteria. Sixty-six percent of these were considered colonized (i.e., contained over 100 cfu/mg comedone material [22]). The majority

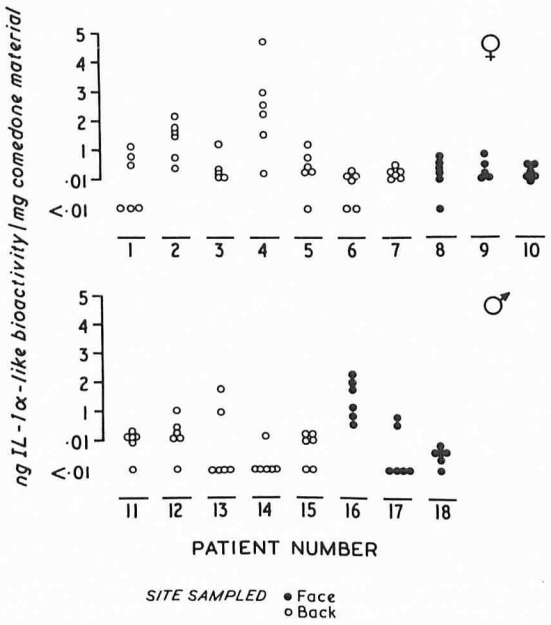

Figure 2. Levels of IL-1α–like bioactivity in individual comedones sampled from the face and back in both sexes. Points show the standardized estimations for comedone extracts following neutralization with anti–IL-1β in the C3H thymocyte assay in the presence of saturating IL-2. In all cases, bioactivity was ablated following neutralization of the comedone extract with anti–IL-1α.

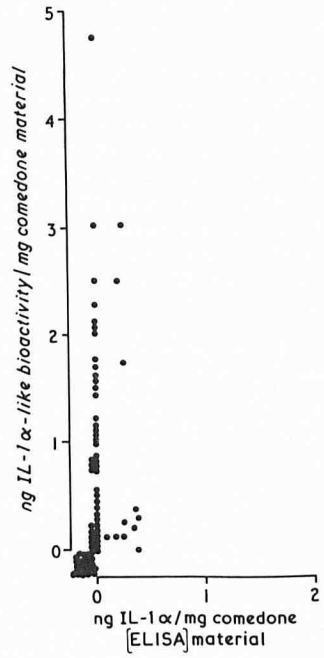

Figure 3. Relationship between immunochemical IL-1α (Quantikine ELISA) and IL-1α–like bioactivity in the 108 comedones obtained from the face (6) and back (12) of 18 untreated acne patients (10 male, 8 female).

of the isolates were *P. acnes I,* with 53 comedones containing only this species of *Propionibacterium.* Seventy-eight percent of comedones contained staphylococci, but only 58% were considered colonized (contained over 100 cfu/mg comedone material). The only true comedone resident was *S. epidermidis.* Thirty-five comedones contained only *S. epidermidis,* with up to four biovars identified in individual samples. *Malassezia* spp. were isolated from 68% of comedones. Of these, only 49% were considered colonized. Forty-two comedones contained the "triad" of microorganisms, three were sterile, seven were monocultures of propionibacteria, seven were monocultures of *Malassezia,* and four were monocultures of staphylococci.

Association Between IL-1α–like Bioactivity, Comedone Weight, and Microorganisms As IL-1α–like bioactivity was present in the majority of comedones, it was important to determine the relationship between this cytokine and microbial density. Spearman's rank correlation coefficients were determined between IL-1α–like bioactivity (pg/comedone) and propionibacteria ($\log_{10}$ cfu/comedone), staphylococci ($\log_{10}$ cfu/comedone), *Malassezia* spp. ($\log_{10}$ cfu/comedone), total microbes ($\log_{10}$ cfu/comedone), and comedone weight (mg). The correlation coefficients were low (0.194, 0.150, 0.209, 0.285, and −0.004, respectively), indicating that a linear correlation between IL-1α–like bioactivity and a particular microbial genus, or indeed total microorganisms or comedone weight, did not exist. There was, nevertheless, a relationship between IL-1α–like bioactivity and the lower limit of total microbial density readily observed on the scatter diagram (Fig 5).

DISCUSSION

This study was based on the premise that non-inflamed lesions may develop into inflamed lesions in acne vulgaris. The hypothesis was that pro-inflammatory cytokines might have a role to play in the development of the initial inflammatory lesion. Thus, levels of IL-1α, IL-1β, and TNFα were estimated in open comedones. Open, rather than closed, comedones were analyzed despite the observation that closed comedones are more likely to precede inflammatory lesions [12]. The choice of open comedones was inevitable for one reason: closed comedones are difficult to remove relatively non-traumatically. It was important in this study that no pro-inflammatory cytokines were released as a result of sampling or derived from the blood.

The measurement of cytokines in biologic fluids is problematic.

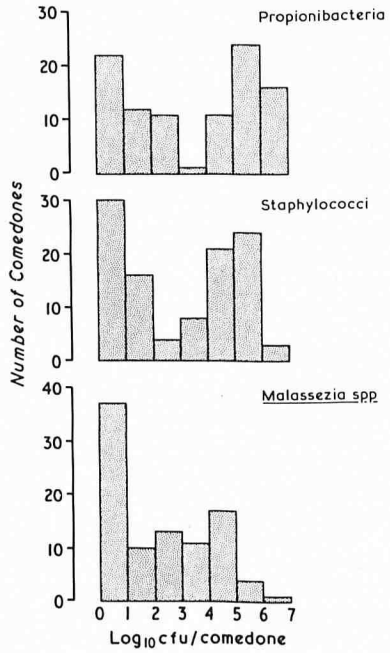

Figure 4. Distribution of microbial population densities among open comedones. The theoretical lower limit of sensitivity was 4 cfu/comedone. Comedones containing over 100 cfu were considered colonized [22].

Table I. Summary of Data for Pro-Inflammatory Cytokine Levels in Open Comedones

	Number Tested	Percent Positive	Range (pg Cytokine/mg)[a]	Mean ± 95% Confidence Limits
IL-1α (bioassay)	106[b]	76	23–4765	648 ± 183
IL-1α (ELISA)	108	10	119–427	284 ± 58.2
IL-1β (bioassay)	108	0		
IL-1β (ELISA)	108	24	12–103	48 ± 10.1
TNFα (bioassay)	108	2.8	61–820	388 ± 969
TNFα (ELISA)	108	4.6	156–390	272 ± 107

[a] Comedone weight range of 0.1–3.9 mg (mean ± 95% confidence limits of 0.83 ± 0.10).
[b] A few samples were lost during processing.

Many factors need to be considered, such as the specificity of the assay, cytokine stability, antagonism or synergism by contaminating cytokines, and the possible presence of cytokine inhibitors [23]. The levels of bioactive cytokines presented in this study were determined only after extensive preliminary studies had been carried out to validate the bioassays. All reported bioactive levels were confirmed after neutralization with specific rabbit polyclonal antibodies. The immunoassays used in this study were not validated "in house." There was no evidence to suggest that serum proteins at high concentrations, capable of interfering in the ELISA (rheumatoid factors, heterophile antibodies, cytokine binding proteins, or active complement [23]) would be present in water-soluble extracts of comedones [24].

The predominant cytokine present in comedone extracts was IL-1. In all comedones in which IL-1 was detected by bioassay, the activity was totally neutralized by anti-IL-1α. This IL-1α–like bioactivity was not detected in the IL-1α ELISA in the majority of cases. This might be explained if the comedone extracts contained material that interfered with the immunoassay but not the bioassay. This would be unusual because most "matrix" effects due to contaminating substances in biologic fluids yield false positive results in cytokine ELISA [23]. Although ELISA may be "blocked" by fresh

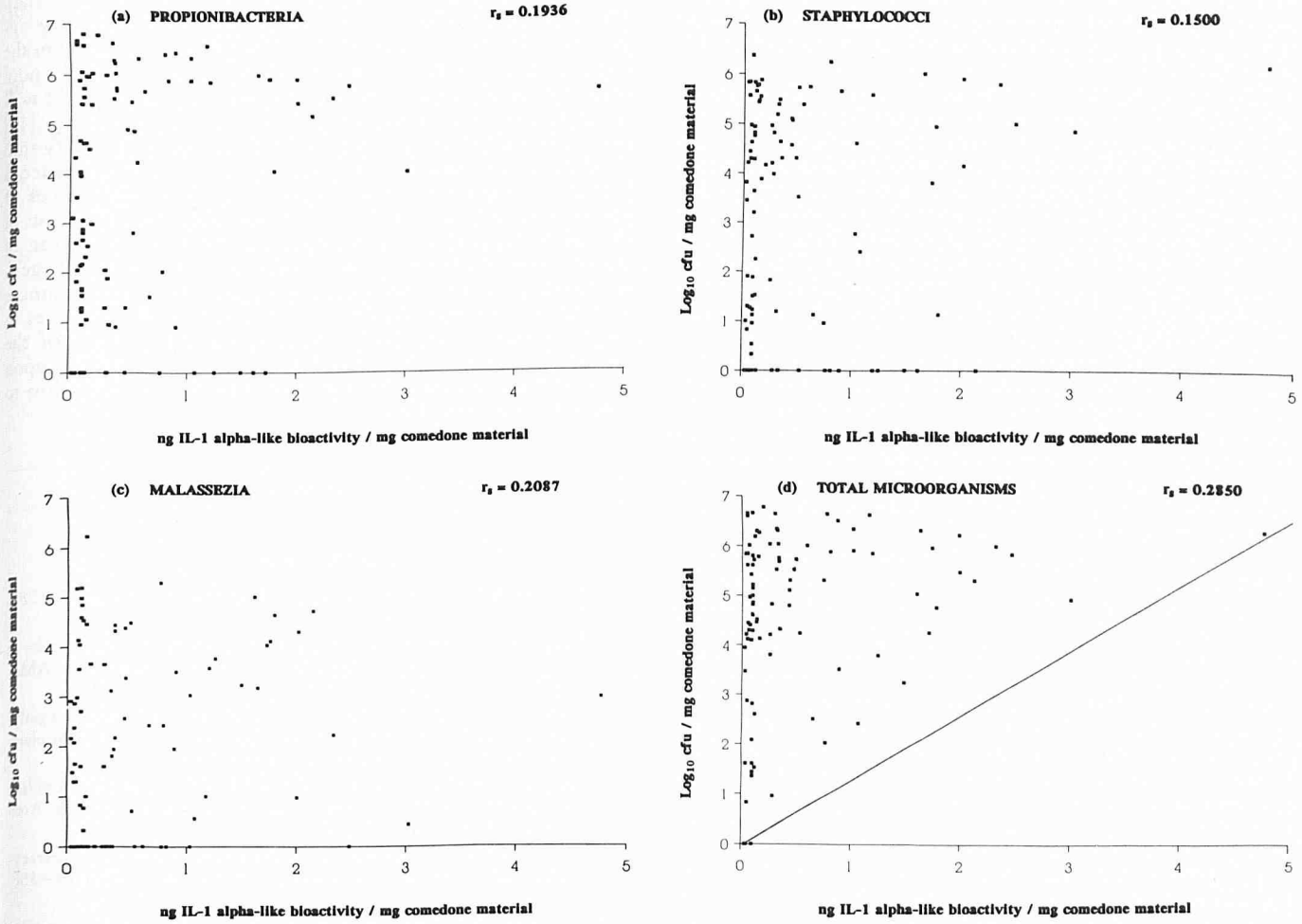

Figure 5. Scatter diagrams for $\log_{10}$ microbial genera and total microorganisms against ng IL-1α–like bioactivity/mg comedone material. The fitted (by eye) line *(d)* depicts a linear relationship between the lower limit of total microbial density and IL-1α–like bioactivity in comedones. This relationship was not due to propionibacteria *(a)*, staphylococci *(b)*, or *Malassezia (c)* when considered independently.

complement [25], previous studies have shown that if any complement was present within the comedones, it would have been rapidly consumed [26]. The evidence suggests that the antibodies used in the ELISA failed to detect comedonal IL-1α-like material. Thus, comedonal IL-1α-like bioactivity may not be epitopically identical with monocyte-derived IL-1α, the source of the material for the ELISA development.

In contrast, IL-1β-like bioactivity was undetectable in 108 comedone extracts. Immunochemical IL-1β was, however, detected in 24% of the comedones by ELISA. This suggests that IL-1β was unstable in comedones, that comedones contained an IL-1β inhibitor, or that IL-1β was cleaved by proteinases (possibly of microbial origin), leaving fragments still capable of being detected in the ELISA.

The results for IL-1 in acne comedones are generally in agreement with previous investigations demonstrating IL-1 in normal human epidermis. It has been suggested that biologically active and immunoreactive IL-1 species in normal human epidermis might derive from different pools [27]. Heel stratum corneum [28,29] contains large amounts of IL-1 bioactivity, as do epidermal samples obtained by heat separation, suction blistering, and keratome slicing [30]. The major IL-1 species in keratome slices from normal skin was similar to IL-1α, as determined by bioassay and antibody neutralization [31]. In heel stratum corneum and chamber fluid samples, the IL-1 has been shown to be a pI 5.0 species, neutralized by anti-IL-1α antibodies [32]. To our knowledge, the IL-1α-like bioactivity present in normal human epidermis has not been measured using commercially available IL-1α ELISA. Other workers have reported IL-1β in epidermal samples by bioassay and chromatography [27,28,33]. An immunoreactive but biologically inactive species of IL-1β has been demonstrated in extracts of psoriatic skin lesions [31].

The cellular origin of the IL-1α-like bioactivity and IL-1β immunoreactive species in acne comedones reported here is not known and was not investigated in this study. The most likely source was the ductal keratinocytes. Both normal and malignant human keratinocytes have been reported to contain mRNA indistinguishable from monocytic IL-1α and IL-1β mRNA as determined by an S1 nuclease protection assay [34]. In culture, keratinocytes have been shown to produce 2–4 times more mRNA for IL-1α than IL-1β and constitutively secrete bioactive IL-1, which was neutralized by an antibody against IL-1α and IL-1β, but not by an antibody specific for only IL-1β [34].

As suggested by Camp et al [32], the IL-1 bioactivity present in comedones should be referred to as "IL-1α-like" because it is not yet possible to confirm the identify of keratinocyte-derived "IL-1α" and the monocyte-derived cytokine. The total lack of correlation between bioassay and immunoassay results in this study suggests possible differences in the epitopes expressed by the two species despite the reported inability to distinguish mRNA for IL-1α in keratinocytes and monocytes [34].

TNFα was not a major constituent of comedone extracts, with bioactive TNFα only being confirmed in 3% of comedones; its source was not known and was not investigated. As both mononuclear phagocytes and human keratinocytes have been shown to secrete TNFα upon stimulation [15], either of these cell types could have been the source. There is no evidence that keratinocytes constitutively secrete TNFα or that TNFα is produced by keratinocytes in vivo. Given the substantial nature of this study it was likely that a few of the comedones isolated were in fact early inflammatory lesions, with signs of inflammation being undetectable by visual examination. Infiltration of the dermis surrounding the lesions by mononuclear phagocytes may have accounted for the small percentage of comedones containing TNFα.

The profile of microbial densities within acne comedones was generally in agreement with previous studies [26,35]. Only three of the comedones contained undetectable microflora and it was of interest that these contained no detectable cytokines. Although there was no correlation between either propionibacteria, staphylococci, or *Malassezia* spp and IL-1α-like bioactivity, there was an interesting association between the lower level of microbial density and IL-1 α-like bioactivity. The reason for this relationship is unclear. A direct effect of microorganisms on the level of IL-1α-like bioactivity cannot be dismissed on the basis of high microbial density in the absence of IL-1α-like bioactivity. If microorganisms stimulated IL-1α-like bioactivity to be produced, a linear correlation might not be evident if in some comedones, the IL-1α-like material was rendered biologically inactive by microbial proteinases. Another explanation was that the relationship was indirect. The microenvironment of comedones may have differed due to other unknown factors, with microenvironment 1 favoring high microbial density but not IL-1α-like bioactivity, microenvironment 2 favoring high microbial density and high IL-1α-like bioactivity, and microenvironment 3 favoring neither.

What is the relevance of this study to the understanding of the development of inflammatory acne lesions? There is little doubt that epidermally derived IL-1α-like bioactivity is a pro-inflammatory cytokine, as studies carried out by Camp et al [32] have clearly demonstrated that the autologous epidermally derived material is capable of inducing visible erythema 4 to 9 h after intradermal injection. Moreover, autologous IL-1α-like bioactivity induced mixed leucocyte perivascular infiltrates with significant increases in numbers of T-helper cells, monocytes/macrophages, and neutrophils in human volunteers [32]. These effects were achieved with 10 and 60 IU of skin chamber fluid-derived IL-1α-like material. Ten international units of IL-1α are approximately equal to 100 pg of human recombinant IL-1α (NIBSC). Nevertheless, 58% of the comedones analyzed in this study contained pro-inflammatory levels of IL-1α-like bioactivity (over 100 pg/mg comedone material). Under normal conditions, epidermal IL-1α-like bioactivity appears to be biologically unavailable [36,37]. However, in acne, the majority of open comedones may clearly represent a "dermal" pool of this pro-inflammatory cytokine. As spongiosis of the follicle wall is a feature of early inflammatory change in acne comedones [13] this could lead to leakage of comedone IL-1α-like bioactivity into the dermis. According to the unifying concepts regarding cutaneous inflammation put forward by Kupper [38], the consequences of pro-inflammatory IL-1 within the dermis would be activation of dermal microvascular endothelial cells, selective accumulation of antigen-non-specific mononuclear cells, and initiation of antigen-independent cutaneous inflammation. In acne, this might promote an amplification phase via antigen-dependent T-cell responses to other comedone components. The intensity and duration of the subsequent specific cell-mediated response would depend upon many factors, including the degree of individual sensitization to their cutaneous microflora.

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ANNOUNCEMENT

The 8th International Steglitz Paediatric Surgical Congress, "Endoscopical Surgery in Children," will be held December 4 and 5, 1992, in Berlin, Germany at the University Medical Centre Steglitz, Berlin. The Chairman is Prof. Dr. J. Waldschmidt. For information contact PD. Dr. F. Schier, Kinderchirurgie, Universitat-Klinikum Steglitz, Hindenburgdamm 30, 1000 Berlin 45, Tel (030) 798 4181 or 798 2909, Fax (030) 798 4141.